Environmental Engineering Research

Environ. Eng. Res. 2013 December,18(4) : 235-239
Research Paper
http://dx.doi.org/10.4491/eer.2013.18.4.235
pISSN 1226-1025 eISSN 2005-968X

Performance of *Chlorella vulgaris* for the Removal of Ammonia-Nitrogen from Wastewater

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Abstract

In the present investigation, the efficiency of *Chlorella vulgaris* (*C. vulgaris*) was evaluated for the removal of ammonia-nitrogen from wastewater. Eight different wastewater samples were prepared with varied amounts of NH_4-N concentrations from 15.22 to 205.29 mg/L. Experiments were conducted at pH 7.5 ± 0.3, temperature 25°C ± 1°C, light intensity 100 μE/m²/s, and dark-light cycles of 8–16 hr continuously for 8 days. From the results, it was found that NH_4-N was completely removed by *C. vulgaris,* when the initial concentration was between 5.22–25.24 mg/L. However, only 50% removal was obtained when the NH_4-N concentration was 85.52 mg/L, which further decreased to less than 32% when the NH_4-N concentration exceeded 105.43 mg/L. The further influence of nitrogen on chlorophyll was studied by various NH_4-N concentrations. The maximal value of chlorophyll *a* (Chl *a*) content was found to be 19.21 mg/L for 65.79 mg/L NH_4-N concentration, and the maximum specific NH_4-N removal rate of 1.79 mg/mg Chl *a*/day was recorded at an NH_4-N concentration of 85.52 mg/L. These findings demonstrate that *C. vulgaris* could potentially be employed for the removal of NH_4-N from wastewater.

Keywords: *Chlorella vulgaris*, Microalgae, NH_4-N removal, Wastewater treatment

1. Introduction

Nitrogen is a one of the most vital nutrients for aquatic plants and algae. However, an excessive concentration of nutrients (containing nitrogen) will stimulate aquatic plant and algal growth and will cause serious pollution problems. In order to prevent these problems, we should handle the nitrogen as a nutrient resource rather than a pollutant that only has to be disposed off. Biological treatment using microalgae is one of the potential treatments to reduce the nitrogen, where the nitrogen is used as a nutrient for the microorganisms.

Many algal species, especially the family of *Chlorella genus*, are found to be tolerant to organic pollutants and could rapidly colonize the given nutrients, such as nitrogen, phosphorus, and organic compounds [1]. The advantages of using algae for this purpose include low operational costs; the possibility of recycling assimilated nitrogen and phosphorus into algae biomass as a fertilizer, avoiding sludge handling problems; and finally, the discharge of oxygenated effluent into water bodies. Moreover, this process is not associated with carbon, as is usually required for nitrogen and phosphorus removal, which is an additional advantage for the treatment of secondary effluents. Microalgae require nitrogen, phosphorus, CO_2, and light for autotrophic growth [2]. Additionally, microalgae are very sensitive to the combined effect of high NH_4-N concentrations and high pH values, because NH_4-N uncouples the electron transport in photo system II and competes with H_2O in the oxidation reactions leading to O_2 generation [3]. In a line, Azov and Goldman [4] observed a significant decrease in the efficiency at high pH (i.e., pH 8) and NH_3 concentrations (i.e., 2 mM) in an algae-containing pond. Similarly, Munoz et al. [5] reported a complete inhibition of *Chlorella sorokiniana* at an NH_3/NH_4^+ concentration of 15 mM at pH 8.7, during the photosynthetically oxygenated treatment of 2 g/L of acetonitrile, in a 50-L column photobioreactor. However, effective use of NH_3-tolerant microalgae can improve the stability of this process. Ogbonna et al. [6] reported no significant effect on the growth of *C. sorokiniana*, even at 22 mM NH_3, whereas *Spirulina platensis* was almost inhibited at very low concentrations of NH_3 (i.e., 11 mM).

One limitation in employing an algal system as the secondary treatment process is the presence of high concentrations of ammonia and urea in raw wastes, especially those discharged from the livestock and food industries, which inhibit algae growth and physiological activity [7]. However, it has been noted that the studies undertaken previously were mainly focused on the effects of N-deficiency, and competitive interaction between nitrate and ammonia uptake at low N level. Relatively little information is available on ammonia removal using different ammonia concentrations in real wastewater treatment by *Chlorella vulgaris* (*C. vulgaris*).

Based on the aforementioned reasons, in the present investigation we have aimed to explore and examine the efficiency of *C.*

Received December 11, 2012 **Accepted** July 13, 2013

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vulgaris in the removal of ammonia-nitrogen from wastewaters containing varied concentrations of ammonia-nitrogen.

2. Materials and Methods

2.1. Microalgae Cultures, Medium and Chemicals

Cells of *C. vulgaris* (FC-16) were cultured in Jaworski's Medium in deionized water, with LED lamps at an ambient temperature. Jaworski's Medium is composed of 4.0 g $Ca(NO_3)_2 \cdot H_2O$, 2.48 g KH_2PO_4, 10.0 g $MgSO_4 \cdot 7H_2O$, 3.18 g $NaHCO_3$, 0.45 g EDTAFeNa, 0.45 g $EDTANa_2$, 0.496 g H_3BO, 0.278 g $MnCl_2 \cdot 4H_2O$, 0.20 g $(NH_4)6Mo7O_{24} \cdot 4H_2O$, 0.008 g Cyanocobalamin, 0.008 g thiamine HCl, 0.008 g biotin, 16.0 g $NaNO_3$, and 7.2 g $Na_2HPO_4 \cdot 12H_2O$ per 200 mL. The cultures were incubated at a constant temperature of 25°C ± 2°C and continuous light intensity of 100 μE/m²/s for 15 days. Cultures of *C. vulgaris* (FC-16) ranged in size from 3–8 μm and were round in shape.

2.2. Characterization of Wastewater

The raw wastewater was obtained from the preliminary sedimentation of a sewage plant at Gangneung, Korea. Table 1 shows the characteristics of the wastewater used throughout our investigation.

The analyzed raw wastewater was noted to be favorable for treatment with microalgae and removal of the available nutrients. An excess ratio of chemical oxygen demand, nitrogen, and phosphorus (i.e., 100:20:2) for this wastewater is recommended for nutrient removal in activated sludge plants. The biochemical oxygen demand (BOD_5) and total phosphorus (TP) and BOD_5 and PO_4 ratios were found to be reasonably high. Similarly, the PO_4 and TP ratio was at its higher range for municipal wastewater. Lastly, calcium, potassium and manganese were not limiting for biological wastewater treatment, and iron was naturally present in the wastewater.

2.3. Experimental Design and Batch Cultivation Method

To eliminate bacteria and protozoa, the wastewater samples were sterilized by autoclaving for 30 min. The experiments were conducted using a batch reactor operation with 1 L conical flasks. At the beginning of each series of experiments, 500 mL of wastewater was inoculated to the flasks with pre-cultured *C. vulgaris*. To evaluate the efficiency of *C. vulgaris* in the removal of ammonia-nitrogen from wastewaters, eight different fractions were prepared with varied concentrations of ammonia-nitrogen. The experimental design adopted is represented in Table 2.

The initial cell density was 1×10^6 cells per milliliter for each experimental set up. The initial chlorophyll *a* (Chl *a*) concentration was kept constant at 2.5 ± 0.5 mg/L, throughout the experiments. The NH_4-N concentration was varied from 15.22 to 205.29 mg/L. The entire experiment was conducted at a neutral pH (7.5 ± 0.3), constant temperature of 25°C ± 1°C, a light intensity of 100 μE/m²/s, and a dark-light cycle of 8–16 hr for 8 days. Raw wastewater was used for this experiment.

2.4. Analytical Methods

The pH values of the cultures were measured intermittently and maintained a constant value of pH (7.5 ± 0.3) by the addition of sterilized and diluted NaOH or HCl.

Dry-weight estimations do not exclusively monitor the amount of algae, because bacteria and zooplankton may add to the biomass. Because only algae contain chlorophyll, the estimation of this pigment was a reliable though elaborate method in algae biomass computation. Depending on the algal strain examined, acetone, ethanol, or diethyl ether was used to extract the pigment from the separated algal cells. In some cases, brief heating was required to achieve complete pigment extraction. After that, the cell debris was removed by centrifugation or filtration, and the extract was protected from light to avoid bleaching of the pigments.

The Chl *a* concentration in the extract was calculated, by reading the absorbance (A) of the pigment extract in a spectrophotometer at a given wavelength against a solvent blank by using Eq. (1) as follows [8]:

$$\text{Chl } a \text{ (mg/L)} = (16.5 \times A_{665}) - (8.3 \times A_{650}) \qquad (1)$$

The experiment was conducted for a maximum of 8 days contact. The specific rate of NH_4-N removal (R_s) was also estimated, using the known Eq. (2):

$$R_s = R/(\text{Chl } a)_o \qquad (2)$$

where $(\text{Chl } a)_o$ stands for the initial concentration of Chl *a* at the time t_o. A specific form of Chl *a* was used in oxygenic photosynthesis.

NH_4-N concentration was measured by ion chromatograph (Metrohm AG, Herisau Switzerland), and the analysis was performed according to the standard methods described elsewhere [9].

Table 1. Characteristics of the raw wastewater

Parameter	Average concentration (mg/L)	Variation (mg/L)
BOD_5	159.63	125.32–180.56
TCOD	270.35	203.18–326.45
TP	6.23	5.07–7.58
PO_4	4.04	3.09–5.15
TN	55.33	37.53–63.24
NH_4-N	11.30	8.80–13.45

BOD_5: biochemical oxygen demand, TCOD: total chemical oxygen demand, TP: total phosphorus, TN: total nitrogen.

Table 2. Characteristics of the experimental design

Fraction	NH_4-N concentration (mg/L)		
	Initial	Increased	Total
Run 1	5.22 ± 0.5*	10	15.22
Run 2		20	25.24
Run 3		40	45.20
Run 4		60	65.79
Run 5		80	85.52
Run 6		100	105.43
Run 7		150	155.38
Run 8		200	205.29

*For all runs (1 to 8).

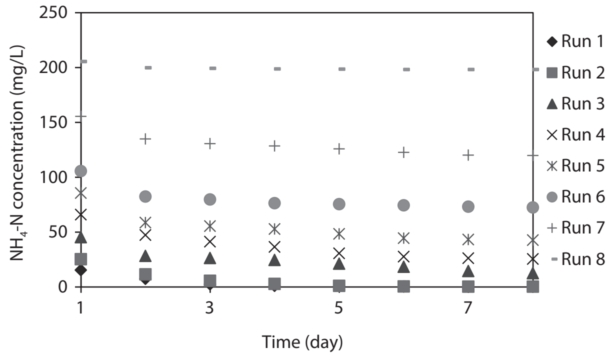

Fig. 1. Processes of NH_4-N removal for different initial NH_4-N concentrations at 8 days.

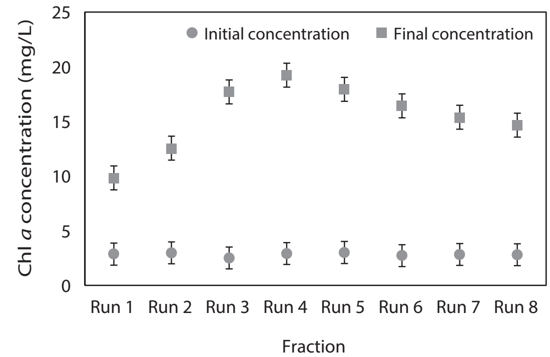

Fig. 2. Variation of chlorophyll (Chl) *a* content with various initial NH_4-N concentrations.

3. Results and Discussion

3.1. Efficiency of NH_4-N Removal

The variation of NH_4-N concentration as a function of time with various initial NH_4-N concentrations is depicted in Fig. 1.

The maximum NH_4-N removal efficiency was obtained after 8 days, and the values were found to be 99.61%, 99.52%, 72.32%, 61.32%, 50.22%, 31.31%, 22.99%, and 3.59% for Runs 1–8, respectively. NH_4-N was completely removed by *C. vulgaris,* when the initial concentration was between 15.22–25.24 mg/L. However, the NH_4-N removal was approximately 50% at an NH_4-N concentration of 85.52 mg/L, and the NH_4-N levels further decreased to less than 32% at the NH_4-N concentration taken beyond 105.43 mg/L. Only 3.59% of the removal efficiency was obtained for the 200 mg/L concentration of NH_4-N. Further, it was observed that the NH_4-N removal efficiency obtained after 2 days was 93.69% for Run 1, 78.49% for Run 2, 41.94% for Run 3, 37.36% for Run 4, 35.23% for Run 5, 24.56% for Run 6, 16.02% for Run 7, and 3.04% for Run 8. The reported NH_4-N removal efficiencies varied, depending on the media composition and environmental conditions, such as the initial nutrient concentrations, light intensity, light/dark cycle, and algae species [10]. The NH_4-N removal efficiency achieved in this study was higher, compared to that of other studies; an average of 72% nitrogen removal was reported for *C. vulgaris* from 3–8 mg NH_4-N/L containing diluted ethanol and citric acid production effluent [11]. Martinez et al. [12] reported over 97% nitrogen removal by *Scenedesmus obliquus* for the initial concentration of 27.4 mg N/L. Olguin [13] obtained a maximum of 96% NH_4-N removal by *Spirulina* in an outdoor raceway as a result of treatment with 2% diluted anaerobic effluents from pig wastewater containing almost the same amount of nitrogen as in the experiment carried out by Martinez et al. [12]. Nevertheless, few reports showed higher or more efficient NH_4-N removal, even at higher concentrations of nitrogen. Shi et al. [14] investigated the effect of the initial nitrogen and phosphorus concentrations on the nutrient removal performance of the algae *Botryococcus braunii* from secondary treated piggery wastewater. The culture was able to consume the available NO_3-N completely, i.e., up to 510 mg/L within 6 days of batch operation. Aslan and Kapdan [2] investigated the batch kinetics of nitrogen and phosphorus removal from synthetic wastewater by *C. vulgaris*. This study showed that a 21.2 mg/L concentration of NH_4-N was removed, using the microalgae *C. vulgaris*.

3.2. Chl *a* and Specific NH_4-N Removal Rate

Nitrogen is the major constituent of proteins, chlorophyll, and enzymes involved in photosynthesis. Therefore, nitrogen affects the photosynthesis of microalgae. The nitrogen absorbed by *C. vulgaris* mostly includes NO_3-N and NH_4-N, and their uptake, deposition, and assimilation in *C. vulgaris* are different. Chlorophyll is an extremely important biomolecule that is critical in photosynthesis, and that allows plants to absorb energy from light. The function of the vast majority of chlorophyll (up to several hundred molecules per photosystem) is to absorb light and transfer that light energy by resonance energy transfer to a specific chlorophyll pair in the reaction center of the photosystem [15]. Chl *a* ($C_{55}H_{72}O_5N_4Mg$) is important in the energy phase of photosynthesis. Two electrons are needed for the electron acceptors to proceed in the photosynthesis process. Within the reaction centers of both photosystems is a pair of Chl *a* molecules that transfer electrons to the transport chain through redox reactions. Chl *a* is a common pigment found in algae. This pigment is what algae use to trap energy from light to promote algal growth. The total Chl *a* content (mg/L) was obtained and is represented in Fig. 2 for the wastewater samples. It was noted that the total Chl *a* content increased gradually with the incubation time in all cultures, with the highest Chl *a* content recorded at day 15 (data not presented).

Fig. 2 clearly demonstrates that the final Chl *a* content of the culture significantly increased from Run 1 to Run 4. However, it decreased gradually beyond Run 5, exceeding 85.52 mg/L of total NH_4-N (Fig. 2). This result suggests that at low NH_4-N concentrations, Chl *a* formation was limited by NH_4-N supply, while excessive NH_4-N concentration does not favor the additional synthesis of Chl *a*. The maximal value of Chl *a* content was 19.21 mg/L for Run 4. These results indicate that *C. vulgaris* is effective in removing NH_4-N concentration at Run 4 (minimal removal efficiency of 60% NH_4-N). In this study, the removal efficiency of NH_4-N up to Run 5 was less than 50%.

The batch data was further utilized to discuss the kinetics of NH_4-N removal. The initial NH_4-N removal rate was used to determine the coefficients. The removal rate (R) was calculated for these wastewater samples, and the obtained values are represented in Table 3.

The maximum NH_4-N removal rate was found to be 5.37 mg/L/day for Run 4. Run 4 sample was obtained 2.8 times and 5.8 times higher than that of Run 1 and Run 8, respectively. The

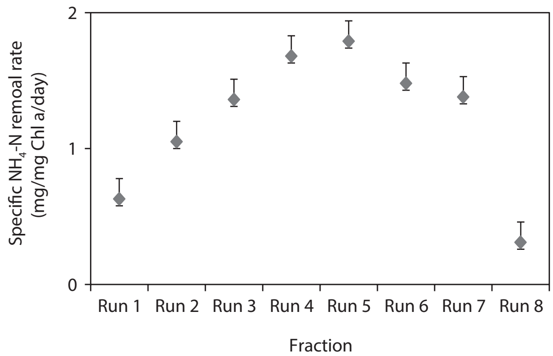

Fig. 3. Specific NH_4-N removal rates for *Chlorella vulgaris*.

NH_4-N removal rates were similar to those obtained previously by other related studies. The nitrogen removal rate by *C. vulgaris* was reported to be 5.44 mg/L/day [15]. However, the lower removal rate was reported as 3.4 mg N/L/day of *Chlorella pyrenoidosa* [16]. Akpor and Muchie [17] also reported somewhat lower removal rates (i.e., 3.36–3.60 mg NH_4-N/L/day, 1.2–3.6 mg NH_4-N/L/day, and 1.2–3.12 mg NH_4-N/L/day by *Aspidisca*, *Trachelophyllum* and *Peranema*, respectively). In contrast, Park et al. [18] reported a substantially higher removal rate of 83 mg N/L/day, upon using the suspended growth culture *Scenedesmus* sp. Similarly, Wang and Lan [19] reported maximal removal rate of 43.7 mg/L/day at 140 mg N/L for *Neochloris oleoabundans*.

The initial cell density was 1×10^6 cells per milliliter, and the initial Chl *a* concentration was kept constant at 2.5 ± 0.5 mg/L throughout the experiments. The specific NH_4-N removal rate for varying NH_4-N concentration was calculated and is depicted in Fig. 3.

NH_4-N absorbed by *C. vulgaris* can be directly used, but the absorbed NO_3-N cannot be used until it is deoxidized to NH_4-N, and the processes consume energy and reducing power. Therefore, NH_4-N can be utilized rapidly at the early stage, which is in favor of chloroplast synthesis and can promote photochemical efficiency. The maximum specific NH_4-N removal rate was found to be 1.79 mg/mg Chl *a*/day for Run 5. At higher concentrations of NH_4-N (after Run 5), the removal rates decreased gradually. These results indicated that the physiological activity of *C. vulgaris* was reduced by high NH_4-N concentrations. The first is that excessive NH_4-N can damage photosynthesis organs and decrease photochemical efficiency, the other is that excessive NH_4-N can markedly increase the ability of chloroplasts to dissipate the excessive energy. So they cannot efficiently utilize the photon energy absorbed by pigments for photosynthesis. NH_4-N partly replacing NO_3-N decreases the consumption of energy and reducing power, while NO_3-N partly replacing NH_4-N relieves metabolic disorder induced by the excessive NH_4-N, and makes the physiological metabolism in *C. vulgaris*. The NH_4-N removal rates, rather than specific removal rates, were reported in most of the previous studies. Aslan and Kapdan [2] reported the specific NH_4-N removal rate from 0.6 to approximately 0.9 mg/mg Chl *a*/day for the NH_4-N concentrations between 60 to 125 mg by *C. vulgaris*.

Table 3. NH_4-N removal rate at various NH_4-N concentrations using *Chlorella vulgaris*

Fraction	NH_4-N removal rate (mg/L/day)
Run 1	1.90
Run 2	3.14
Run 3	4.09
Run 4	5.37
Run 5	5.04
Run 6	4.13
Run 7	4.46
Run 8	0.92

4. Conclusions

In this study, the potential of *C. vulgaris* for the removal of various concentrations of ammonia-nitrogen from wastewaters using batch reactor operations was evaluated. From the results, it was found that NH_4-N was completely removed by *C. vulgaris* in the initial concentration range 5.22–25.24 mg/L. Therefore, *C. vulgaris* is more suitable for domestic wastewater treatment, than that of industrial wastewater treatment containing NH_4-N. The maximal value of Chl *a* content was found to be 19.21 mg/L for 65.79 mg/L NH_4-N concentration. However, the maximum specific NH_4-N removal rate was found to be 1.79 mg/mg Chl *a*/day, with the initial NH_4-N concentration of 85.52 mg/L. At higher concentrations of NH_4-N (after Run 5), the removal rates gradually decreased. From the results, it can be concluded that the physiological activity of *C. vulgaris* was reduced by high NH_4-N concentrations. The plausible reason for this is that first, excessive NH_4-N can damage photosynthesis organs and decrease photochemical efficiency. Further, excessive NH_4-N can markedly increase the ability of chloroplasts to dissipate the excessive energy.

Acknowledgments

This research was supported by the Basic Science Research Program through the National Research Foundation of Korea, which is funded by the Ministry of Education, Science and Technology (No. 2012-0002804/2013006899).

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